

The IgE repertoire in PBMCs of atopic patients is characterized by individual rearrangements without variable region of the heavy immunoglobulin chain bias

Annick Lim, MSci,^{a,b} Stephan Luderschmidt, MD,^c Anke Weidinger, MD,^c Christina Schnopp, MD,^c Johannes Ring, MD,^c Rüdiger Hein, MD,^c Markus Ollert, MD,^{c,d} and Martin Mempel, MD^{c,d,e} Paris, France, and Munich, Germany

Background: Patients with atopic diseases are characterized by high levels of specific IgE production. However, little is known about the composition of their B-cell repertoires.

Objectives: We sought to analyze the complete PBMC-derived IgE repertoire and to compare clonal expansions between different patients.

Methods: We have analyzed the IgE-bearing B-cell receptor repertoire in highly atopic patients (>1000 IU/mL) using quantitative RT-PCR, complementarity determining region 3 spectratyping, and sequence analysis. Three representative patients were additionally followed during anti-IgE therapy.

Results: Atopic patients exhibited 100 to 1000 times more IgE-specific transcripts than control individuals. These patients used a variable region of the heavy immunoglobulin chain (VH) ϵ repertoire highly similar to their IgM and IgG repertoires, with preference of VH3b, VH4, VH3a, and VH1 segments. Each patient harbored individual clonal expansions, most probably as correlation of allergen-specific IgE production. Common expansions within the complementary determining region 3 shared by several individuals with similar sensitization patterns were found in spectratyping analysis. However, these antigen-driven expansions showed differences on the sequence level. In omalizumab-treated patients the clinical improvement was paralleled by a clear increase in the ratio of IgG/IgE transcripts.

Conclusion: The IgE repertoire in atopic patients follows the VH use patterns seen for other immunoglobulins and seems to

preferentially recruit individual rearrangements rather than public expansions.

Clinical implications: The detailed analysis of the IgE B-cell repertoire is highly suitable to follow changes in IgE uses during different therapy modalities. (J Allergy Clin Immunol 2007;119:1016-1025.)

Key words: B cells, atopic eczema, IgE repertoire, omalizumab

The peripheral B-cell receptor repertoire is shaped by a combination of somatic hypermutation, affinity maturation, or both in the transition of premature to mature B cells, adapting the antibody response to the antigen of interest. In atopic individuals a substantial part of the peripheral B-cell compartment has undergone class switching to the production of IgE caused by genetic susceptibility of the immune system, which is followed by an enhanced production of IL-4 and IL-13, which in turn favors the deviation of a given immune response to a T_H2 type with high IgE production.¹

For IgE class switching, the rearrangement of the heavy chain locus can either present in a single-step rearrangement process from the μ to the ϵ locus or in a multistep process, inducing first B-cell clones using the γ 1, γ 2, or γ 4 segment, followed by a final excision step that fuses the ϵ locus to the preformed variable region of the antibody.² Although the regulatory cytokine IL-10 seems to favor the switch to these IgG (especially IgG4) subclasses, lack of IL-10 in the presence of high IL-4 levels then leads to enhanced IgE switching.^{3,4}

The IgE repertoire in atopic individuals is not constant and depends on several factors, such as reboost of specific B cells by allergen contact, humoral (IL-4 and IL-6) and cellular (CD40–CD40 ligand) costimulation,^{5,6} the turnover time of IgE antibodies,⁷ and the proportion of membrane-bound and secreted IgE molecules.⁸ Whereas cell-bound IgE has a relatively long half-life, serum IgE is degraded rapidly, with a half-life of between 6 hours and 2.5 days.⁹

Thorough analysis of the B-cell compartment is hampered in several ways. First, B cells carrying IgE on their surfaces are relatively rare in PBMCs, accounting for only approximately 1% of all CD19⁺ and CD20⁺ B cells in the

From ^aUnité de Recherche et d'Expertise Immunité anti-virale, Biothérapie et Vaccins, Paris; ^bINSERM U668, Institut Pasteur, Paris; ^cthe Department of Dermatology and Allergy, Biederstein, Technical University Munich; ^dthe Clinical Research Division of Molecular and Clinical Allergotoxicology, Technical University Munich; and ^ethe Division of Environmental Dermatology and Allergy, GSF National Research Center for the Environment and Health/Technical University Munich, Munich.

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Reprint requests: Martin Mempel, MD, Klinik und Poliklinik für Dermatologie und Allergologie, der TU München, Biedersteiner Str. 29, D-80802 München, Germany. E-mail: m.mempel@lrz.tum.de.

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Abbreviations used

CDR: Complementarity determining region
 CH: Constant region of the heavy immunoglobulin chain
 VH: Variable region of the heavy immunoglobulin chain

periphery. Numbers of IgE-positive plasma cells are probably even lower, accounting for less than 0.1% of plasma cells, as defined by the surface marker CD138 or the high expression of CD27.¹⁰ Second, unlike for the variable T-cell receptor chains, no antibodies reliably recognizing the B-cell receptor–variable region of the heavy immunoglobulin chain (VH) segments have been described. Therefore the exploration of the IgE repertoire in adults has relied on random cloning of selected B-cell populations, followed by sequence analysis of their hyper-variable region. Apparently, individuals with similar sensitization profiles show similar IgE antibody production in their sera.¹¹ Consequently, common traits in the sequences of IgE rearrangements between different individuals have been postulated and have been found, to some extent, by means of cloning analysis.¹² In analogy to the T-cell receptor diversity, these rearrangements can be described as “public” in contrast to unique “individual” rearrangements.¹³ Of interest, some studies showed a preferential rearrangement of the VH5 chain,^{14–16} a variable chain that is used at low percentages for IgM and IgG clones. However, not all studies confirmed this preference.^{17–19} VH5, on the other hand, seems to be preferentially found in B-cell clones that are selected in mucosal tissues.^{20–22} Moreover, cloning of a set of allergen-specific IgE rearrangements in a single atopic individual has revealed a preferential use of VH6,²³ a segment that is also used by only a minority of IgM and IgG clones.^{24,25}

It was therefore our interest to depict the entire IgE repertoire in the periphery of highly atopic patients and to apply this technique to selected individuals undergoing anti-IgE therapy.

METHODS

Individuals

All 14 recruited individuals were seen for atopic eczema consultation in the Department of Dermatology and Allergy, Biederstein, Technical University Munich. All patients were older than 18 years and had severe atopic eczema. A detailed list of the patients' characteristics is given in Table I. All patients provided informed consent for venous puncture and withdrawal of 50 mL of blood.

Included control patients were healthy adult volunteers displaying IgE levels of less than 75 IU/mL.

All patients included in omalizumab therapy (Xolair; Novartis, Nürnberg, Germany) had bronchial asthma and allergic rhinoconjunctivitis in addition to atopic eczema and likewise provided informed consent to be followed by extensive documentation for the course of atopic eczema during treatment. All patients were informed of the off-label use of omalizumab for atopic eczema. For these patients, 150 mg of omalizumab was delivered subcutaneously in 2-week intervals, and total serum IgE levels, the extent of eczema (SCORAD²⁶), and a follow-up photograph were documented.

Omalizumab therapy was carried out for 10 cycles, after which a final evaluation was performed. B-cell repertoire analysis in PBMCs was performed before starting the therapy and between the sixth and eighth cycles of omalizumab administration. Additionally, a patient receiving cyclosporine (3 mg/kg body weight) therapy who responded clinically in a comparable way was included.

Serum immunoglobulin analysis

The anti-human IgG and IgM ELISAs were from Bethyl Laboratories (Montgomery, Tex). IgE levels in serum were measured by using the Immulite 2000 system (DPC Biemann, Bad Nauheim, Germany) and in a second approach by coating omalizumab directly as a capture antibody to the plastic wells and otherwise following a standard sandwich ELISA protocol. This approach carries the advantage that IgE/omalizumab complexes and free IgE can be distinguished, which is not possible with conventional techniques. All tests were evaluated by using human myeloma IgE (Calbiochem, San Diego, Calif) and myeloma IgE/omalizumab complexes in defined ratios.

IgE, IgG, and IgM repertoire analysis with quantitative RT-PCR

At first presentation and under the condition that patients had avoided any systemic treatment for the last 4 weeks, 50 mL of EDTA blood was withdrawn for PBMC preparations.

RNA was prepared from the obtained cells by using the RNeasy kit from Sigma (Deisenhofen, Germany). RNA was converted into cDNA according to standard protocols by using Superscript II (Invitrogen, Carlsbad, Calif), oligo dT primers (Eurogentech, Paris, France), and RNasin (Invitrogen).

PCR reactions were carried out by combining a primer and a specific fluorophore-labeled probe for the constant region (CH ϵ , CH μ , or CH γ), with one of 8 primers covering the different VH1 through VH7 chains. Reactions were carried out with the Taqman 7300 device (Applied Biosystems, Foster City, Calif) and standard reagents from Applied Biosystems. Because all primers were chosen to exhibit identical affinities, it was then possible to calculate the relative distribution of IgE, IgM, and IgG transcripts and the relative use of the different VH families. Details of the primers and the calculation of the relative transcriptions are filed as European and US patent (Repertoire determination of a lymphocyte B population, WO 2005/059176 A1) and can be obtained through the Pasteur Institute (Paris, France).

Immunoscope analysis

In a second approach PCR products were then subjected to runoff reactions with a nested fluorescent primer specific for the constant region (F $\epsilon\epsilon$, F $\epsilon\mu$, or F $\epsilon\gamma$) for 5 cycles. The fluorescent products were separated and analyzed on an ABI-PRISM 3730 DNA analyzer. The size and intensity of each band was analyzed with Immunoscope software.²⁷ Fluorescence intensity was plotted in arbitrary units on the y-axis, whereas the x-axis corresponds to complementary determining region (CDR) 3 length in amino acids.

Sequence analysis of B-cell receptor transcripts

PCR products were cloned into pCR4Blunt-TOPO vector (Invitrogen, Cergy Pontoise, France). Sequencing was carried out with the Big Dye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems) and analyzed on an ABI PRISM 3730 DNA analyzer. Sequences corresponding to the CDR-3 regions and VH mutations of the corresponding sequences were extracted and analyzed by using gcg software (version 10.3; Accelrys, Inc, San Diego, Calif) and Taps1.1 software (written by Emmanuel Beaudoin).

TABLE I. Patient characteristics

Patient no.	Sex/age (y)	Total IgE (IU/mL)	Diagnosis	Specific IgE levels (>3.5 kU/L)
1	M/64	10,669	AE	HDM, HD, B, DD, CD, TP, MW
2	M/39	13,674	AE, RCA, AB	TP, B, HDM, CD, N, P, W
3	F/44	18,732	AE, AB	HDM, CD, HE, CF, W, C, TP, L, B, MW
4	F/47	6240	AE, RCA, AB	HDM, CD, TP, B, MW
5	F/66	20,048	AE, RCA, AB	HDM, CD, TP
6	M/30	6262	AE	HDM, CD, W, C, TP, L, B
7	M/42	34,957	AE, RCA, AB	HDM, CD, TP, CF, AF, W, L, B, MW, R, P, T, CH
8	F/47	23,565	AE, RCA, AB	HDM, CD, HE, MP, CF, W, L, B, MW
9	F/38	3107	AE, RCA, AB	CD, W, TP, L, B, MW
10	F/63	4352	AE, RCA, AB	HDM, HD, DD, CD, TP, MW, B, L, N, W, P
11	F/48	2914	AE, RCA, AB	HDM, CD, TP, B, MW
12	F/21	1018	AE, RCA	C, TP, B
13	F/24	13,970	AE, RCA, AB	HDM, AS, CH, HD, DD, CD, TP, MW, B, N, HE, MP, CF, W, P, L, C
14	M/44	20,193	AE, RCA, AB	HDM, CD, P, N, T, W, R, C, TP, B, MW

F, Female; M, male; AE, atopic eczema; RCA, rhinoconjunctivitis allergica; AB, asthma bronchiale; HDM, house dust mite; CD, cat dander; HE, hen egg; MP, milk protein; CF, codfish; W, wheat; L, latex; B, birch; MW, mugwort; HD, horse dander; DD, dog dander; TP, timothy pollen; N, hazelnut; P, peanut; AS, *Aspergillus* species; CH, *Cladosporium herbarum*; MP, milk protein; C, celery; R, rice; T, tomato.

RESULTS

Quantitative use of Fc ϵ , Fc μ , and Fc γ transcripts and proportional use of variable segments within the heavy chains (VH segments)

In control individuals the percentage of IgE transcripts among total transcripts of IgM, IgG, and IgE generally was found to be very low, accounting for less than 0.001% of all analyzed immunoglobulin-specific transcripts. In general, the vast majority of immunoglobulin transcripts were represented by IgM-specific transcripts (>98%) and, to a much lesser extent, IgG-specific transcripts (1% to 2%, Fig 1).

Among the 8 different variable chains, we found a dominant use for the VH3b, VH3a, VH4, and VH1 segments together with lower use of VH2, VH5, VH6, and VH7, regardless of the type of immunoglobulin under investigation (Fig 2).

Analysis of IgE transcription in 15 highly atopic individuals revealed higher IgE transcription within their PBMCs, occasionally reaching up to 4% of all immunoglobulin-specific mRNA molecules (Fig 1). Among the VH segments used by atopic patients within their IgE molecules, we did not find particular preferences compared with the use of VH chains among IgM and IgG molecules. These preferentially used VH segments were VH3b, VH3a, VH4, and VH1 and thus did not differ from the distribution of VH chains in healthy individuals. Of note, we did not find use of either VH5 or VH6 above the ratio expected from healthy donors or from the distribution of the IgM or IgG rearrangements (Fig 2).

CDR-3 spectra in CH ϵ -VH ϵ rearrangements

Having established the differential use of VH chains for IgE expression, we sought to identify possible dominant expansions within the different CH ϵ -VH combinations. This approach revealed multiple peak-like profiles within

the CDR-3 length analysis suggestive of the expansion of clonal populations. A comparative analysis of VH1, VH3a, VH3b, and VH4 (the 4 preferentially used VH segments among CH ϵ rearrangements in atopic subjects) is given in Fig 3. This analysis shows that the relative distribution of the preferred CDR-3 length is highly different between patients and does not suggest common patterns.

IgE repertoire in atopic patients is composed of individual expansions

Interestingly, among several patients sharing the same pattern of sensitizations, we found rearrangements that were used with similar profiles between different patients, such as the CH ϵ -VH3a rearrangement, which was compared between patients 3, 4, and 7.

To identify possible public rearrangements between patients, PCR products were cloned and randomly sequenced. In each patient at least 45 different VH3a-CH ϵ rearrangements were sequenced and compared (see Figs E1 and E2 in this article's Online Repository at www.jacionline.org). This experiment revealed intraindividual dominant sequences that were isolated several times, most probably as signs of clonal expansions (eg, the 8-amino-acid rearrangement in patient 4, which was isolated 21 times).

However, although most of the obtained sequences shared common traits between each other and between different patients, we did not observe identical hyper-variable regions, suggesting individual selection and affinity maturation patterns rather than public rearrangements.

By comparing the sequences in detail, we found a high degree of somatic mutations together with affinity maturation. For example, of the 58 sequences of the VH3a-CH ϵ rearrangement in patient 3, 54 were derived from the germline sequence of V3-73, of which 25 showed signs of mutation; 3 were derived from V3-49, which were all mutated; and 1 was derived from germline sequence

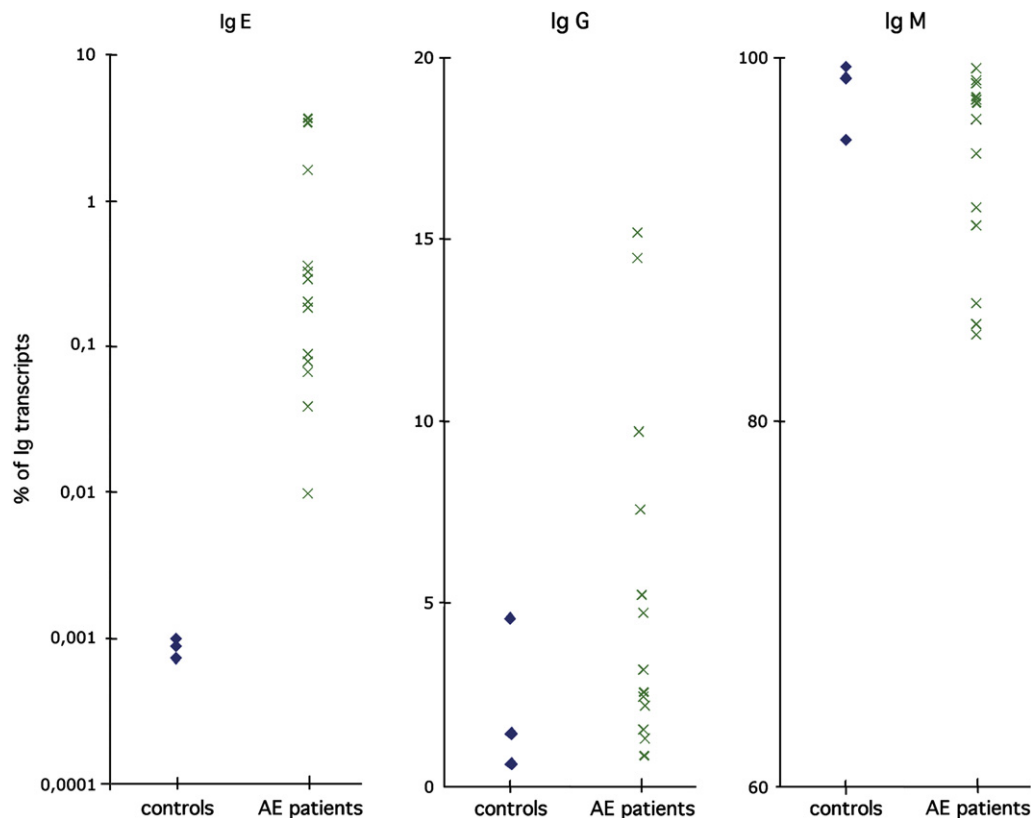


FIG 1. All included atopic patients showed an enhanced transcription of IgE compared with that seen in nonatopic control subjects. Note the logarithmic scale on the y-axis, indicating that some patients yielded up to 4% of IgE among the combined totality of IgM, IgG, and IgE transcripts. IgM transcripts represented the majority of immunoglobulin transcripts, ranging from 84% to 99%, together with IgG percentages of between 1% and 15%. AE, Atopic eczema.

V3-49, which was unchanged. In total, of 58 sequences, only 30 (51.7%) carried germline configuration. Within the VH region FR3, we found a frequency of somatic mutation of between 3% and 25% per 100 bp.

Analysis of the quantitative IgE repertoire in atopic patients undergoing anti-IgE therapy

To further apply the analysis of the B-cell repertoire, we have followed 3 patients during anti-IgE therapy. All 3 patients had very high total serum IgE levels before starting omalizumab therapy (>10,000 IU), and all patients had extended eczema, as documented by SCORAD values of greater than 50 (Fig 4, A). In all patients, 10 cycles of 150 mg of subcutaneous omalizumab injection were administered at 14-day intervals. Of the 3 patients, 2 (patients 13 and 7) responded rapidly to the treatment with a clear and lasting improvement of their clinical scores, whereas patient 2 remained unchanged with regard to his SCORAD value (Fig 4, A).

As expected, the relatively low dose of anti-IgE had no substantial influence on total or free serum IgE levels at any point of the observation interval (Fig 4, B). However, in patients 13 and 7 we observed dramatic changes of the immunoglobulin repertoire on the mRNA levels.

Both patients had strongly increased IgE mRNA transcripts before therapy, with almost 4% (IgG 4.6%) in patient 13 and 0.1% (IgG 3.4%) in patient 7 of all immunoglobulin-specific transcripts. In the course of anti-IgE treatment, however (between the sixth and the eighth injection of 150 mg of omalizumab), we observed a surprising increase of IgG transcripts, together with a decrease of IgE mRNA in both patients, which resulted in a percentage of IgG transcripts among all immunoglobulin-specific mRNA of 15.3% (IgE 0.4%) in patient 13 and 11.8% (IgE 0.03%) in patient 7. Patient 2, who clinically did not respond to anti-IgE treatment and who showed preclinical values of 0.08% IgE and 1.49% IgG transcripts, increased to 0.28% IgE and 2.5% IgG transcripts after the seventh omalizumab treatment cycle (Fig 4, C).

As control for omalizumab-specific results, we included patient 14, who was treated with cyclosporine and who clinically responded in a comparable way (decrease in SCORAD value of 25). In PBMCs from this patient, we observed an increase in the percentage of IgE-specific transcripts from 0.2% to 0.4%, an increase of IgM-specific transcripts from 85.3% to 97.3%, and a decrease in IgG-specific transcripts from 14.5% to 2.4% (Fig 4, C).

Relative transcription of VH chains

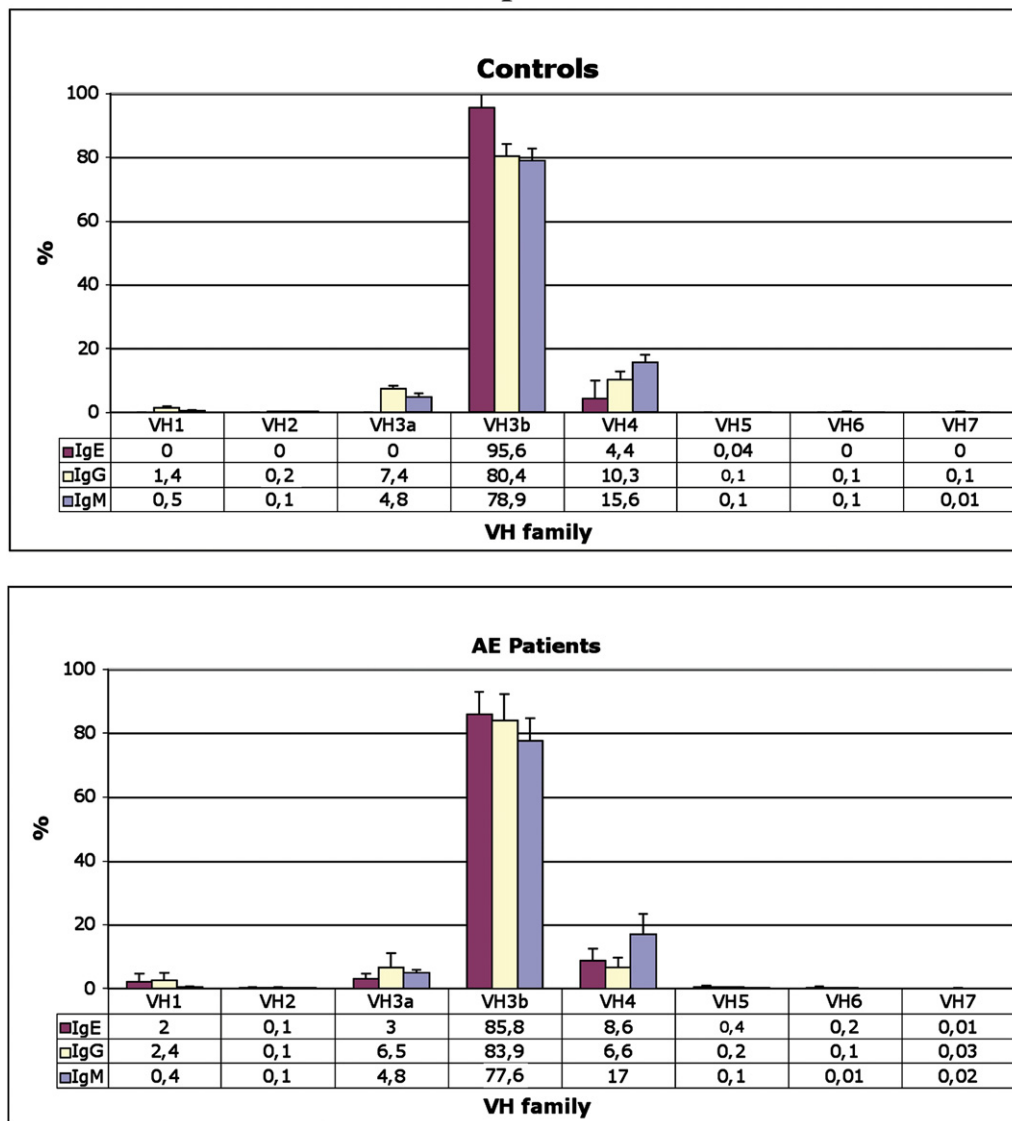


FIG 2. Distribution of the various VH chains among the total immunoglobulin repertoire by means of quantitative PCR shows that IgE antibodies from atopic patients used VH chains in an almost identical percentage as IgM and IgG antibodies in healthy and atopic individuals. For each included individual, the dominant VH segment was VH3b, followed by VH4, VH3a, and VH1, with the latter varying between patients. AE, Atopic eczema.

CDR-3 spectratyping during omalizumab therapy

Analysis of CDR-3 profiles of the VH3b-CH ϵ rearrangements in omalizumab-treated patients showed that for patient 2, no major differences occurred in both the VH3b-CH ϵ and the VH3b-CH γ rearrangements, whereas patient 13 showed a broader use of rearrangements within the VH3b-CH γ segment, together with a virtually unchanged VH3b-CH ϵ spectrum during therapy. Patient 7, in contrast, revealed a broader use of VH3b-CH γ rearrangements, together with a clearly reduced repertoire of the VH3b-CH ϵ combination (Fig 4, D).

DISCUSSION

In the present study we have analyzed the peripheral IgE heavy chain repertoire in highly atopic individuals. These patients not only showed strongly increased serum levels of IgE antibodies but also increased transcription of the CH ϵ chain of the immunoglobulin locus. The percentages of IgE-specific transcripts were in general 100 to 1000 times higher in atopic than in control individuals and thereby reflected relatively accurately the increase of IgE molecules in the serum. Importantly, our technique is not suitable to differentiate between changes

IgE Immunoscope

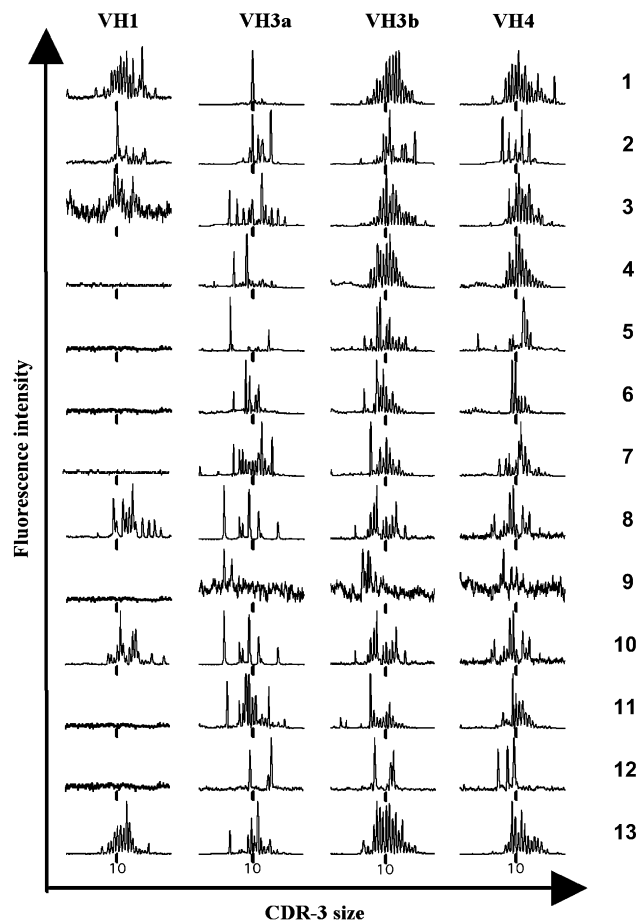


FIG 3. IgE immunoscopy (CDR-3 spectratyping) for the preferentially used VH segments VH1, VH3a, VH3b, and VH4 is shown for all included patients, with individual expansions suggestive of antigen (allergen) stimulation. The line (CDR-3 size) marks the average size of 10 amino acids for any rearrangement. Although some common patterns are found (eg, the 10-amino-acid peak of the CH ϵ -VH3a rearrangement between patients 1 and 12 or the 8-amino-acid peak of the CH ϵ -VH3b rearrangement shared by patients 12 and 7), all patients display rather individual patterns of their CDR-3 profiles, arguing for individually selected repertoires.

in immunoglobulin-specific mRNA caused by changes in the amount of mRNA per cell or changes caused by cell numbers expressing the respective immunoglobulin-specific mRNA. However, because FACS staining for absolute numbers of IgM⁺, IgG⁺, and IgE⁺ CD19⁺ B cells maximally differed by a factor of 5 between atopic and control individuals (data not shown), we think that the number of transcripts per cell plays a crucial role.

Our approach revealed only a moderate quantitative association for immunoglobulin transcripts and serum immunoglobulins. This fact can be explained by several reasons. First, we analyzed percentages of immunoglobulin transcripts and not absolute values, which of course do not reflect the quantification of immunoglobulin in serum, for which absolute values are calculated. Second, we analyzed IgE mRNA-producing cells within PBMCs. A large part of the antibody production, however, is most probably caused by cells not circulating in the blood.

These are long-living plasma cells, which are located in bone marrow,^{28,29} and plasma cell clones, which are induced in distinct body compartments (eg, mucosal tissue of various origin). For these cells, mRNA was not analyzed, although IgE secreted from these cells probably is found in serum.³⁰ Third, the half-life of circulating IgE is highly variable, depending on the association to Fc ϵ receptors and the rate of internalization after activation through the high-affinity receptor Fc ϵ RI.⁸

Our analysis revealed that, at least in our adult collective, the distribution of VH chains followed the use seen for other immunoglobulins in the periphery. The preferential use of the families VH3b, VH4, VH1, and VH3a has already been described in IgM and IgG repertoires.^{31,32} These families have the highest number of submembers,³¹ somehow arguing for a random selection. Because for patients with atopic eczema and patients with atopic rhinitis a preferential use of VH5 and VH6 has been

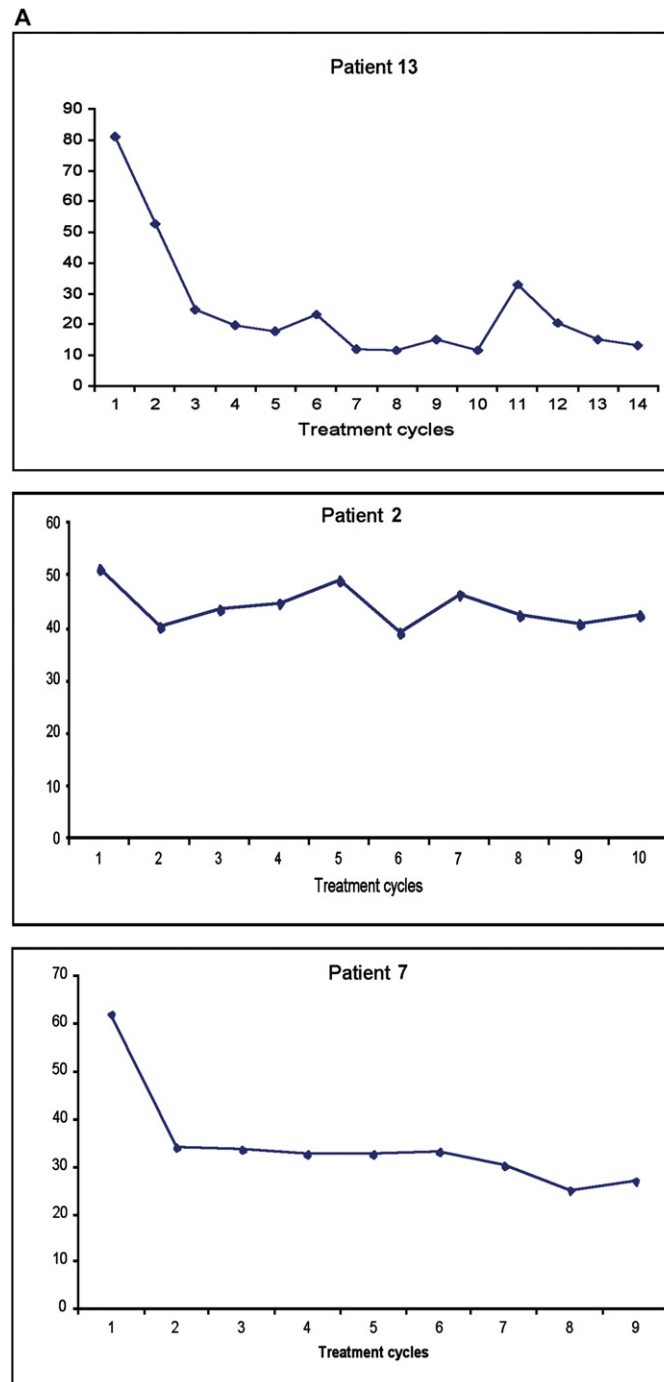


FIG 4. A, Images show the SCORAD values before and after 10 cycles of omalizumab. Clinical images are posted in the Online Repository in [Fig E3. B,](#) Results for the immunoglobulin analysis for all 3 patients receiving omalizumab therapy. The graph for IgE represents the results expressed in micrograms per milliliter and represents unbound IgE. **C,** The 2 patients responding well to omalizumab therapy (13 and 7) clearly downregulated their relative IgE mRNA while in parallel upregulating their relative IgG mRNA. Patient 2 and the cyclosporine-treated patient 14 responded with a slight upregulation of IgE mRNA, together with either downregulated IgG transcripts and upregulated IgM (14) or no significant changes (2). Values are expressed as percentage transcription of the respective immunoglobulin among the combined total transcripts for IgM, IgG, and IgE. *BT*, Before therapy; *DT*, during omalizumab therapy. **D,** CDR-3 profiles for the predominately used rearrangement CH ϵ -VH3b in omalizumab-treated patients. Whereas patient 2 showed virtually no changes in IgE and IgG repertoires, patients 13 and 7 showed broader profiles of their IgG transcripts, together with unchanged (13) or reduced (7) IgE repertoires for this rearrangement. IgM (with the exception of a single peak for patient 7) remained in a Gaussian-like distribution.

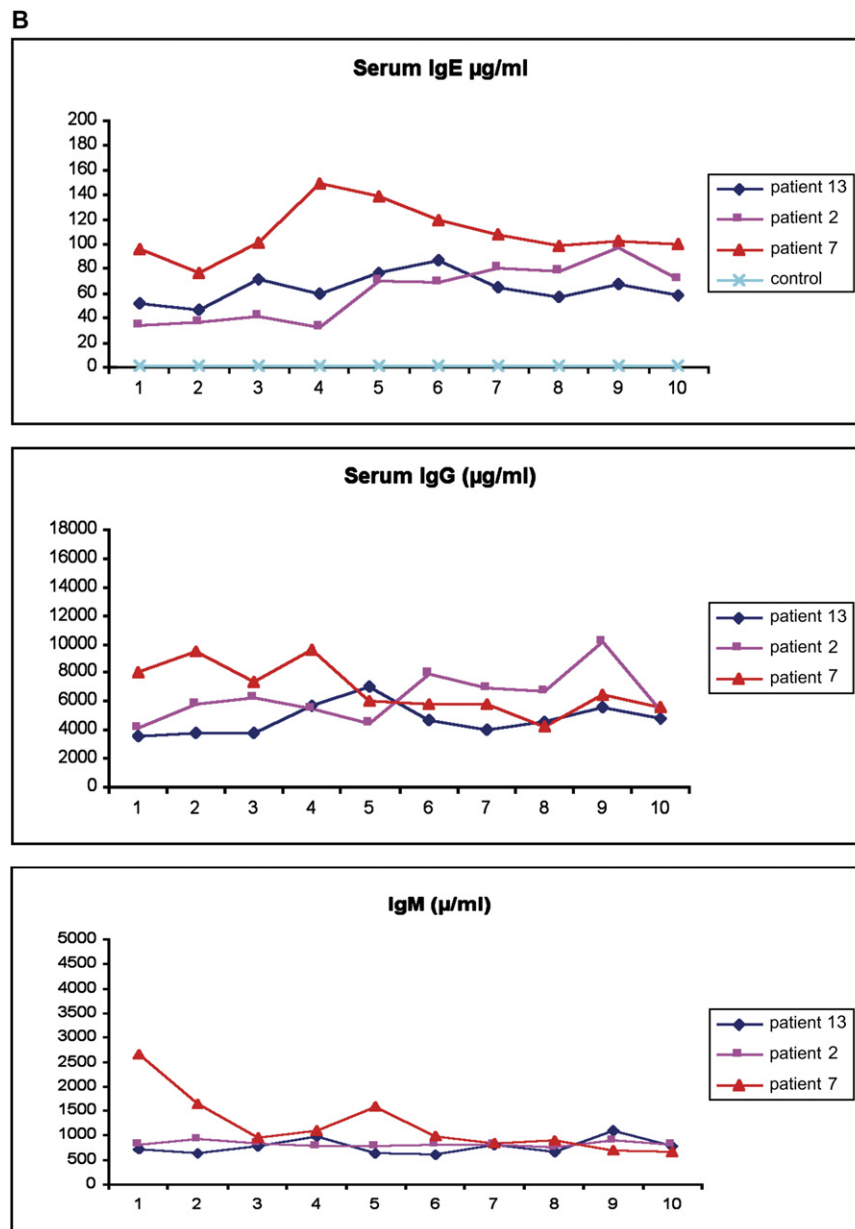


FIG 4. Continued.

described,^{14,16,23} we verified that the lack of VH5/VH6 amplification was not due to a lower affinity of the used primers, thereby neglecting substantial amounts of these IgE transcripts. This hypothesis can be excluded for 2 reasons. First, the quantitative approach was evaluated by means of thorough testing with recombinant plasmids of the various VH segments (including both VH5 families VH5-51 and VH5a), which showed highly similar affinity patterns for all used VH segment-specific primers. Second, our distribution analysis of VH segments in IgG and IgM molecules depicted the numbers given in the literature, with only minute percentages of VH5/VH6 transcripts.

In line with our results, a recent publication on IgE transcripts in the PBMCs of allergic individuals showed use of the VH5 and VH6 segment in the expected range.¹⁹

However, in the case of mucosa-based class-switch recombination, as reported for the nasal mucosa of individuals with pollen allergy, a bias toward VH5 was repeatedly seen, possibly as a result of selection by superantigens.³⁰ Although a final conclusion cannot yet be drawn, it seems that the IgE repertoire in PBMCs shows less bias toward a certain VH family than compartment-specific repertoires. Because we had no access to B-cell populations in tissue, we were not able to compare the PBMC repertoire with local IgE repertoires in our patients.

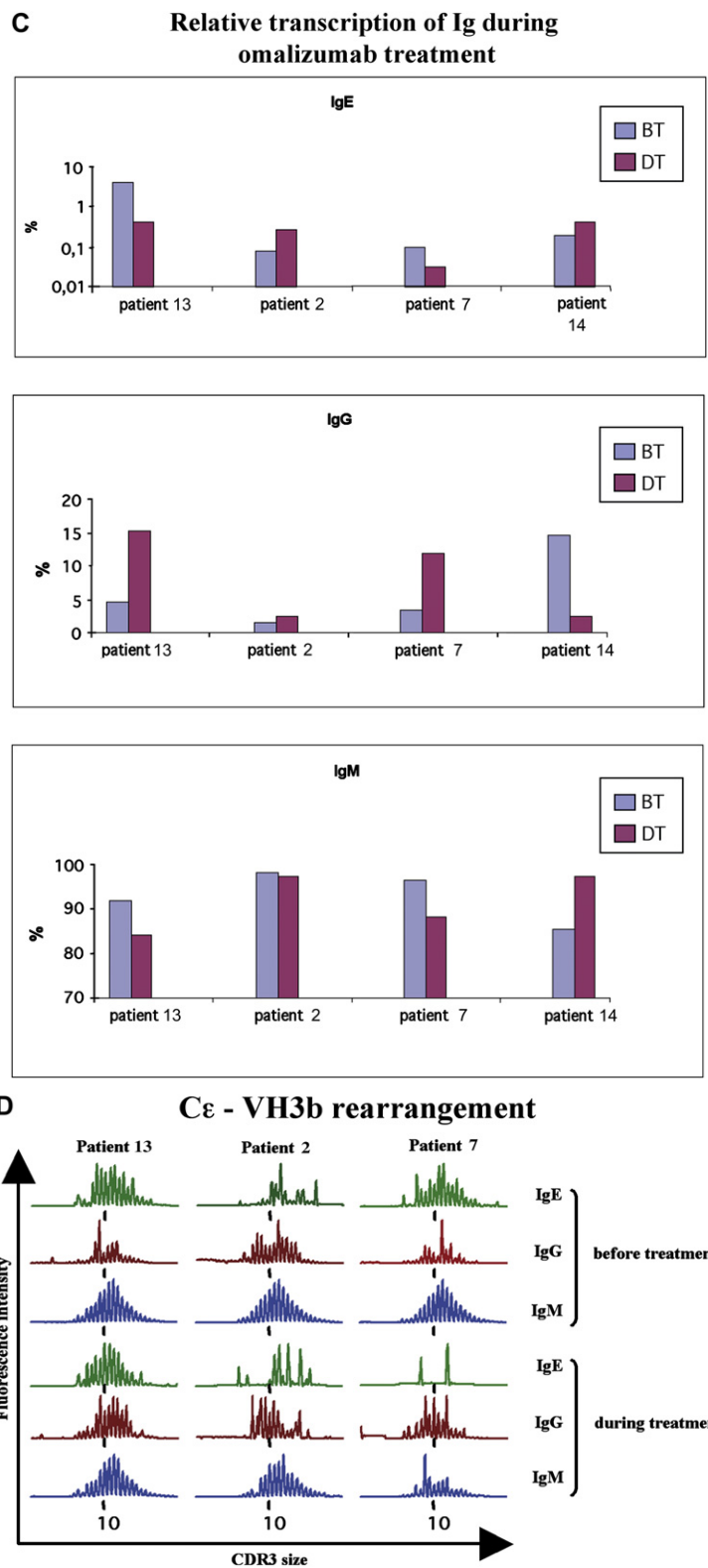


FIG 4. Continued.

Our findings were also complemented by the CDR-3 profiles for the preferentially used VHe segments VH3b, VH4, VH1, and VH3a, all of which showed highly individual patterns without signs of common imprints on the repertoire.

All these results together argue for the concept that in atopic individuals there is no preferential VH segment for any of the immunoglobulins and that in principle, in response to a given antigen challenge, similar repertoires are used for IgM, IgG, and IgE repertoires.

The analyzed sequences and additional 58 sequences for the VH3a-CHε rearrangement in patient 3 showed that IgE sequences in atopic individuals have multiple mutations, which is suggestive of affinity maturation during the process of antigen recognition. Type and level of mutations within the allergen contact site of the antibody further prompted us to look for common patterns within IgE rearrangements between different individuals. Such dominant public rearrangements have been found for T-cell receptor rearrangements between MHC-matched individuals but have never been described for human immunoglobulin molecules. To this end, the dominant VH3a-CHε rearrangements of patients 7, 3, and 4 were sequenced. However, despite an extensive analysis of altogether 177 sequences, we were not able to find matching results between the different patients. This fact rather argues for individual-associated preferences in the fine tuning of IgE antibody responses than for a preferential pattern that is induced by the allergen itself. Of note, the samples analyzed in our study were collected in the absence of a specific allergenic stimulus (eg, provocation tests); however, because the acquisition took place over the range of 6 months, we cannot rule out that spontaneous (eg, seasonal aeroallergens) challenges had an effect on our results.

To apply the technique of the B-cell receptor repertoire analysis to a clinical setting, we followed 3 patients undergoing omalizumab treatment. These patients repeatedly showed IgE levels of greater than 10,000 IU/mL, and thus an appropriate dosage to remove the entire IgE molecules from the serum would have been unrealistic.³³ We therefore decided to apply a relatively low-dose regimen by administering 150 mg of omalizumab subcutaneously every 14 days. Surprisingly, this dosage led to a good clinical response in 2 of 3 patients, together with impressive changes in the IgE and IgG transcripts. In the 2 responding patients, transcripts for IgE were downregulated, together with a clear increase in IgG transcripts. The clinically unresponsive third patient showed a rather unchanged proportion of IgE- and IgG-specific transcripts. In parallel to the findings of overall transcription, we also observed accordant profiles in the CDR-3 spectratyping analysis, showing larger IgG repertoires in the responding patients together with unchanged profiles in the nonresponsive patient. Interestingly, total serum IgE, as well as total serum IgG and total serum IgM, showed no changes in all 3 patients during therapy. One explanation could be the persistence of antibody-producing plasma cells, which are not affected by omalizumab. A

similar phenomenon is seen in patients receiving anti-CD20 therapy, who are completely devoid of B cells for 9 to 12 months but sustain their antibody levels in the periphery.^{34,35}

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CDR3-spectratyping of the C ϵ -VH3a rearrangement

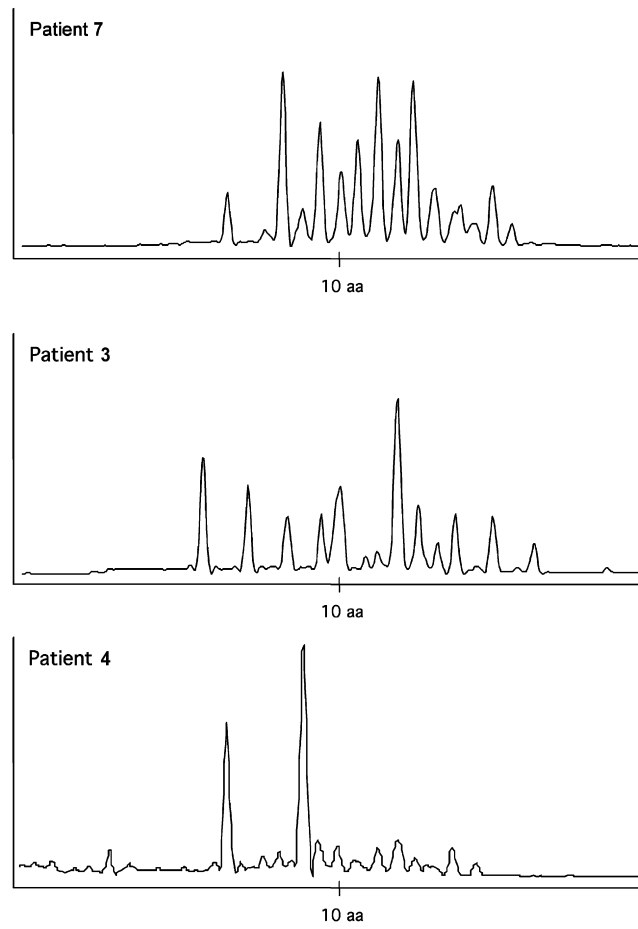


FIG E1. Comparison of the C ϵ -VH3a rearrangements among patients 7, 3, and 4 by CDR-3 spectratyping. All 3 patients show multiple expansions that are shared interindividually (eg, the 7-amino-acid or 13-amino-acid rearrangement).

Patient 7				Patient 3				Patient 4			
VH3a	CDR3	JH		VH3a	CDR3	JH		VH3a	CDR3	JH	
			times isolated				times isolated				times isolated
SLKTEDTGYYVCGD	4 aa EGRQ	VQQGTLVTSSA	2	SLKTEDTGYYCLA	3 aa GRY	WGQGLTVSSA	1				
NLQSDDTAVYYCTT	7 aa GGHYVGS	TQQGTLVTSSA	2								
SLQTDDTAVYYCTT	GGHYVGS	LPGGTVVTVSSA	1	SLKTEDTAVYYCTR	5 aa VGLGS	WGQGIRVTSSA	1				
SMKSDDTGYYCAT	8 aa EMGGHFDY	WGQGLTVTVSSA	1	SLKSDDTAVYYCNR	7 aa PATRDRY	WGQGLTVTVSSA	1	SLKTEDTAVYYCTF	7 aa GSGSFSY	WGQGLTVTVSSA	2
SLKSDETGAYYCAT	EMGWHFYD	WGQGLTVTVSSA	1	SLKTEDTAVYYCTG	EDNSGWY	WGQGLTVTVSSA	1				
SLKSDDTGAYYCAT	EMGWHSYD	WGQGLTVTVSSA	1					SLRAEDTAVYYCAR	8 aa RAWELLDY	WGQGLTVTVSSA	21
SLKSDDTGAYYCAT	EMGWHFYD	WGQGLTVTVSSA	2								
SLNTEDTAVYYCTR	9 aa DSHGYYFDY	WGQGLTVTVSSA	1								
SLRPEDTALVFCAR	TMIVDYFDY	WGQGLTVTVSSA	3	SLKTEDTAVYYCTR	9 aa HVVAGSFD	WGQGLTVTVSSA	9	SLKVEDSAVYFCST	9 aa IVGANILTY	WGQGLTVTVSSA	1
SMKTEDTAVYYCSR	VYPEVGIDY	WGQGLTVTVSSA	1								
SLKTEDTAVYYCTR	GDPVAGIDY	WGRGTLVSVSSA	1								
SLKSEDVAVYYCAR	AGYDHGFDV	WGQGLTVTVSSA	1	SLKTGDTAVYYCYR	10 aa LSPVSGGPDY	WGQGLTVTVSSA	1	RLTTEDTAVYYCGR	11 aa QLYGGYYMDV	WKGTTTVTVSSA	1
SLKTDDTAVYYCTR	GDAVAGIDY	WGQGLTVTVSSA	4	SLKTGDTAVYYCSR	LSPDSGGPDY	WGQGLTVTVSSA	1				
SLKTEDTAVYYCAR	GDPVAGIDY	WGQGLTVTVSSA	2	SLKTGDTAVYYCSR	LSPASGGPDY	WGQGLTVTVSSA	8				
SLKTEDTAVYYCTR	GDAVAGIDY	WGQGLTVTVSLS	1	SLKTGDTAVYYCSQ	HSPGGRGPDY	WGQGLTVTVSSA	1				
SLKTEDTAVYYCTR	GDPVAGIDY	WGQGLTVTVSSA	4	SLKTDDTAVYYCTR	RAVQGGVNDY	WGQGLTVTVSSA	1				
SLKTGDTAVYYCAT	11 aa SSTWYVGDSD	WGQGLTVTVSSA	1	SLKTGDTAVYYCCR	LSPASGGPDY	WGQGLTVTVSSA	1				
SLRAEDTAVYYCAK	DLGGSWLTFDY	WGQGLTVTVSSA									
	12 aa			SLKTEDTAVYYCST	13 aa GLMYYGSGSHFDY	WGQGLTVTVSSA	1	SLKNEDTAVYYCTR	13 aa GSEQTPEYYMDV	WKGTTTVTVSSA	2
SLRAEDTAVYYCA	TESALTPAPFHY	WGQGLTVTVSSA	1	SLKTEDTAVYYCFS	HCELRVCSGGGHL	WGQGLTVTVSSA	4	SLKNEDTAVYYCTR	ASEQSPVYYMDV	WKGTTTVTVSSA	1
SLRAEDTAVYYCAK	DRGAATGTGDF	WGQGLTVTVSSA	1	SLKTEDTAVYYCFQ	HCELRSCSGGGHL	WGQGLTVTVSSA	3	SLKTEDTAVYYCTR	LYDYGNSVPFDI	WGQGTMTVTVSSA	1
SLKTEDTAVYYCTT	ISYYYHSSGPNP	WGQGLTVTVSSA	1	SLKTEDTAVYYCFQ	HCELRFCSGGGHL	WGQGLTVTVSSA	1	SLKTEDTAVYYCTR	LLVIGTVRGHIDY	WGQGLTVTVSSA	1
SLKTEDTAVYYCFG	DIFWSAKIRDDY	WGQGLTVTVSSA	1	SLKIEDTAVYYCST	GVMYVDSGSHLDY	WGQGLTVTVSSA	1				
	13 aa			SLKIEDTAMYYCMG	HCELRSCSGGGHL	WGQGLTVTVSSA	7				
SLKTEDTAVYYCTL	GRAENSYYYGMDV	WGQGLTVTVSSA	4								
SLKTEDTAVYYCTI	PLTGHNGFVDHIDY	WGQGLTVTVSSA	1								
SLKTEDTAVYYCAR	VRSAWQTYWYFDL	WGRGTLVSVSSA	1								
SLKTEDTAVYYCAR	ARSAWQTYWYFDL	WGRGTLVSVSSA	1								
	14 aa			SLKTEDSAIYFCAS	14 aa WTAVTTTSFWYFDL	WGRGTLVTVSSA	1	SLKTEDTAVYYCTR	14 aa PRYCTNGVCNMEV	WGMGTTTVTVSSA	1
SLRAEDTAVYYCAT	KSALTAVAGTPLDF	WGQGLTVTVSSA	1					ILKTEDTAVYYCSR	PRYCTNGVCNMEV	WGI GTTVTVSSA	2
SLRVEDTAVYYCAK	ALVGLNYYYGLDV	WGQGTTVTVSPA	2					LKTEDTAVYFCTR	QYPPIAIVAAGLV	WGQGLTVTVSSA	1
SLSPEDTAVYFCAR	QGREYGGILRGFDY	WGQGLTVTVSSA	1								
SLKTEDTAVYYCTT	GGVYSGS8SVSR	WGQGLTVTVSSA	1								
SLKIEDTAIYFCAR	HPVRGYYYSGMIDV	WGQGTTVTVSSA	3					SLKNEDTAVYYCTR	15aa GALGTETVTLRGVL	WGQGLTVTVSSA	1
SLKTEDTAVYYCTR	RYCSGGTCYANIDY	WGQGLTVTVSSA	4					SLKTEDTAIYYCTR	PGSYCGDCYEDWEDP	WGHGTLTVTVSSA	1
TLKTEDTAVYYCTR	15 aa DQYLYTSRTSYGMDV	WGQGTTVTVSSA	1					SLKSDDTAVYYCAR	PGSDCGDCYEDWEDP	WGHGTLTVTVSSA	3
SPKTEDTAVYYCCR	DLYYYASGSSYGMDF	WGQGLTVTVSSA	1								
SPKPEDTGYYCSR	DQYFYTSSSYGMDV	WGPGATVTVSSA	1	SLKVEDTAVYYCTT	16 aa EVPYCTSGRCWAYFDL	WGCGTLTVTVSSA	1	SLKTEDTAIYYCTR	16aa PGSYCGDCYEDWEDP	WGHGTLTVTVSSA	1
SLKIEDTAVYYCTR	DLYYHPSRTSYGMDV	WGQGTTVTVTVSSA	1					SLKSDDTAVYYCAR	PGSDCGDCYEDWEDP	WGHGTLTVTVSSA	
	17 aa										
SLRAEDTAVYYCAK	ITTDYGDYATRITQYFDY	WGQGLTVTVSSA	2					SLKTEDTAVYYCTR	17aa HLSEYGDYIIYYIYMDV	WKGTTTVTVSSA	4
SLRVEDTAVYYCAK	ITTDYGDYATRITQYFDY	WGQGLTVTVSSA	2					SLKTEDTAVYYCAR	HLSEYGDVYYYIYMDV	WGRGTTTVTVSSA	1
SLRVEDTAVYYCAK	ITTDYGDYVTRITQYFDY	WGQGLTVTVSSA	2					SLKTEDTAVYYCAR	HLSEYGDVYYYIYMDV	WGRGTTTVTVSSA	1
SLRVEDTAVYYCAK	ITTDYGDYVTRITQYFDY	WGQGLTVTVSSA	10					SLKTEDTAVYHCTR	HLTEYGDYIIYYIYMDV	WKGTTTVTVSSA	1
	18 aa							SLKEDTAVYFCSR	HLTEYGDYIIYYIYMDV	WKGTTTVTVSSA	1
SLRSEDTGYYFCAT	VGTLHYYYDRSGYFYFDN	WGQGLTVTVSSA	6					SLKTEDTAVYYCSR	HLSEYGDVYYYIYMDV	WKGTTTVTVSSA	1
SLRSEDTGAYFCAT	VGTLHYYYDRSGYFYFDN	WGQGLTVTVSSA	2								
SLKTEDTAVYYCTT	ALLRYGTGGTWSAFYFD	WGQGLTVTVSSA	1								
SLKTEDTAVYYCSR	DYYGSGNYYMYYYGMDV	WGQGLTVTVSSA	1								
	20 aa										
SLRAEDTAVYYCAR	AKYRAFANYDSSGYDAFDI	WGQGTMTVTVSSA	1								

Sequence alignment of the Cε –VH3a rearrangement

FIG E2. A large-scale sequence analysis of a total of 177 sequences did not reveal identical sequences shared between the different patients. Of interest, specific IgE sensitizations (as found in the serum) included house dust mite, timothy grass pollen, birch, cat dander, and mugwort for all 3 patients.

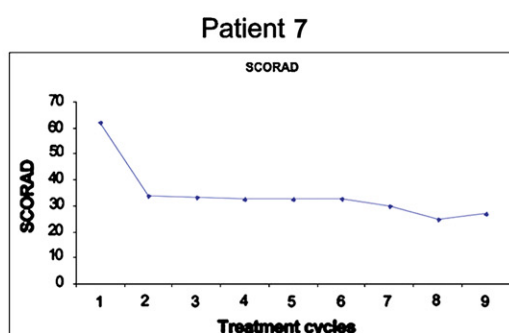
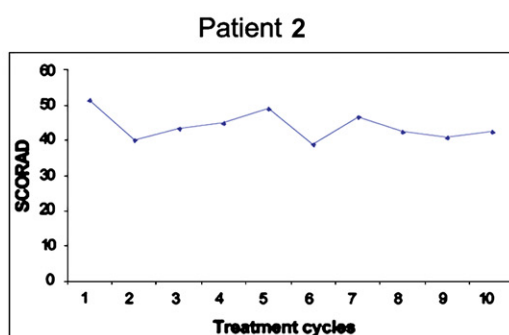
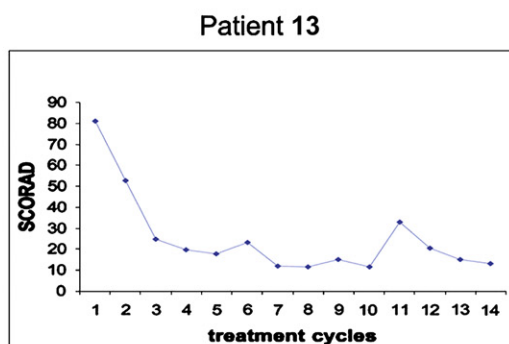


FIG E3. Clinical course of the 3 patients with atopic eczema treated with 150 mg of subcutaneous omalizumab in 2-week intervals. Patient 13 showed a clear amelioration of her symptoms, dramatically reducing her SCORAD value. Patient 2 showed no clinical response, whereas patient 7 also revealed a good clinical response. Scores were taken before every injection, and photodocumentation is shown after 10 cycles of omalizumab treatment.